REPORT

by Assoc. Prof. Dr. Svetlana Milcheva Momchilova -
Institute of Organic Chemistry with Centre of Phytochemistry (IOCCP)-BAS,
Assessor in Scientific Jury appointed by order РД-09-183/26.07.2019,

concerning the materials submitted for the competition for academic position **Associate Professor** at IOCCP-BAS in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.2. Chemical Sciences, scientific specialty Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances,
for the needs of **Laboratory Chemistry and Biophysics of Proteins and Enzymes**,
announced in the State Gazette issue 43/31.05.2019 and on the website of IOCCP-BAS

General presentation of the procedure and the applicant

Only one applicant has submitted documents in the competition, namely **Assistant Professor Dr. Alexandar Konstantinov Dolashki** from Lab. Chemistry and Biophysics of Proteins and Enzymes at IOCCP-BAS. He has presented the documents in due time in accordance with the requirements of the Act for the Development of the Academic Staff in the Republic of Bulgaria (ADASRB), the Regulations for its application, the Regulations of the BAS and the IOCCP-BAS on the conditions and the procedure for the acquisition of scientific degrees and occupation of academic positions.

Assist. Prof. Dr. Alexandar Dolashki has completed his higher education in 2000 at the University of Chemical Technology and Metallurgy – Sofia as a Master of Science Chemical Engineer (specialty Chemical technological processes and systems). In 2005 at the Eberhard Karls University of Tuebingen (Germany), the Faculty of Chemistry and Pharmacy, after successfully passing exams and defending a PhD thesis “Structure, functions and properties of copper-containing proteins: hemocyanins and superoxide dismutase”, he was awarded the degree Doctor (Dr.rer.nat.). In 2006, the Higher Attestation Commission of Bulgaria approved its educational and scientific degree "Doctor" in the specialty 01.05.10 "Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances". Since 2006, Dr. Alexandar Dolashki has been working at IOCCP-BAS.

In the competition for the academic position Associate professor, Dr. Alexandar Dolashki has presented 24 scientific publications, from which:

- 11 as equivalent to habilitation work (indicator V), published in the following ranked journals (SJR quartile): 3 in Q1, 5 in Q2, 2 in Q3 and 1 in Q4. In 8 of these publications Dr. Alexandar Dolashki is the first author and in 2 he is the second;

- 13 other scientific papers (indicator G), of which 4 are in journals with rank Q1, 4 are in Q2, 2 are in Q3, 1 is in Q4 and 1 is in an SJR journal without an impact factor. Indicator G also

contains 4 national patents (protected in 2013, 2016, 2017 and 2019) of co-authors including Dr. Alexandar Dolashki.

The candidate has submitted a list of 25 additional publications beyond the ones pointed above, and a list of 258 citations, of which 176 concern the articles included in this competition (64 for publications of indicator V and 112 for those of indicator G); h-index 10.

Scientific work of Assist. Prof. Dr. Alexandar Dolashki includes also his active participation in 16 national and 14 international projects. He has been a coordinator of 2 national projects and a coordinator of the Bulgarian participants in 3 international ones. The results of his research work have been presented at more than 50 national and international conferences and congresses.

He is the co-author of the university book "The Essence and Biological Application of Mass Spectrometry" (Simelpress, Sofia, 2012) and a Notebook to it.

Thus Assist. Prof. Dr. Alexandar Dolashki exceeds the minimum requirements of the IOCCP-BAS Regulations for the occupation of the academic position of Assistant Professor.

Research activities of the applicant

Scientific contributions of Assist. Prof. Dr. Alexandar Dolashki`s work in the field of bioorganic chemistry presented in this competition have been published in the articles on indicator V, in the following areas:

1. ISOLATION AND CHARACTERIZATION OF THE STRUCTURE AND PROPERTIES OF PROTEINS WITH ONE COPPER ION IN THE ACTIVE SITE (SUPEROXIDE DISMUTASE, SOD). Studies with two fungal strains (*Humicola lutea* and *Aspergillus niger*) have been performed. Experiments with the former have shown that Cu/Zn-SOD exists in both the mitochondrial intermembrane space and cytosol. Cu/Zn-SOD and Mn-SOD were isolated from the latter fungal strain and their molecular masses were determined. The primary structures of Cu/Zn-SOD have been fixed for both strains. The mitochondrial Cu/Zn-SOD of *H. lutea* has been found to be the first identified naturally glycosylated enzyme from a fungal strain, whereas the isolated enzyme from *A. niger* is not a glycoprotein. Its temperature and pH stability were evaluated to be high.

2. ISOLATION AND CHARACTERIZATION OF THE STRUCTURE AND PROPERTIES OF PROTEINS WITH TWO COPPER IONS IN THE ACTIVE SITE (HEMOCYANINS). New hemocyanins from crab *Eriphia verrucosa* and marine snail *Rapana venosa* living in the Black Sea have been isolated and analyzed in detail. Thus, additional information on their structure and properties in molluscs and arthropods was obtained.

3. ISOLATION AND CHARACTERIZATION OF THE STRUCTURE AND PROPERTIES OF GLYCOPROTEINS WITH TREE COPPER IONS IN THE ACTIVE SITE (TYROSINASES). Two bacterial tyrosinases were isolated, purified and analyzed by different methods from bacterial strains

Streptomyces albus and *Laceyella sacchari* that had not been studied for tyrosinase activity previosly. It has been found that in contrast to eukaryotic organisms bacterial tyrosinases are not glycosylated. The enzyme isolated from *S. albus* shows both monophenolase and diphenolase activities.

4. PROTEOMIC ANALYSES OF ANTITUMOR ACTIVITY OF HEMOCYANINS. The growth of human bladder tumor cell lines, CAL-29 and T24, is detected in the presence of *Helix lucorum*, *Rapana venosa*, *Megatura crenulata* hemocyanins and their functional units. It has been suggested that glycosylation of hemocyanins has an important role in their anticancer activity. In addition, for the first time the antimicrobial activities of hemocyanins of the molluscs *R. venosa* and *H. aspersa* were examined. A structural subunit of *H. aspersa* has shown strong antimicrobial activity against Gram-positive bacterial strains *S. aureus* and *Streptococcus epidermidis*, but also against Gram-negative bacterium *Escherichia coli*. It is believed that this subunit has the potential to become a substitute for commonly used antibiotics against which bacterial resistance has been developed.

Assist. Prof. Dr. Alexandar Dolashki has presented his intention about future work, including extension of research and development on peptides and glycopeptides. That is closely related to his participation in two large-scale scientific projects - Center of Competence “Clean Technologies for Sustainable Environment - Water, Waste, Energy for Circular Economy”, and the National Scientific Program “Innovative Low-Toxic Biologically Active Precision Medicine (BioActMed).

Critical comments and recommendations

I have a few trivial remarks about the presentation of some materials in the competition. There are several differences in the data given in the Habilitation Summary on the applicant's scientific contributions, in the Table for fulfilling the requirements for the academic position, and in the list of publications of indicator G. These minor gaps, however, do not decrease the applicant's score to (over)fulfill the requirements for academic position "Associate Professor" and do not affect the excellent impression of his research activity and applied achievements.

CONCLUSION

Assist. Prof. Dr. Alexandar Dolashki is a member of scientific group with an impressive number of prestigious national and international awards for the exploitation and commercialization of scientific results, including the individual PYTHAGORAS 2012 Science Award for a team of business research; the highest prize, statuette and certificates "Inventor of the Year 2012", gold plaque (2014 and 2016), silver medal (2011 and 2015), diplomas and certificates from the annual national exhibition "Inventions, technologies , innovation", etc.

Summarizing the results of scientific activities of Assist. Prof. Dr. Alexandar Dolashki it can be concluded that, in addition to their undisputed importance for practice, they contribute also for extending and deepening of the existing knowledge in field of bioorganic chemistry about the structure and properties of proteins and glycoproteins. This fact, as well as meeting all requirements of ADASRB and Regulations for the occupation of the academic position "Associate Professor" in IOCCP-BAS, gives me a reason for **positive assessment** and to recommend to the Scientific Jury to propose to the Scientific Council of IOCCP-BAS to elect **Assist. Prof. Dr. Alexandar Dolashki** as **Associate Professor** in the professional field 4.2 "Chemical sciences", scientific specialty "Bioorganic chemistry, chemistry of natural and physiologically active compounds".

18.09.2019 г. Signature:

Sofia (Assoc. Prof. Dr. Svetlana Momchilova)